

# The Fourth United States–Japan Meeting on the Toxicological Characterization of Environmental Chemicals

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The fourth joint meeting between scientists from the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina, and the Biological Safety Research Center (BSRC), National Institute of Health Sciences (NIHS), in Tokyo was held 2–3 March 1993, in Research Triangle Park. The purpose of these meetings, held under the auspices of the U.S.–Japan Agreement on Cooperation in Research and Development in Science and Technology, is to exchange information on the toxicological characterization of environmental chemicals of mutual interest (1–3). The emphasis

of the fourth joint meeting was on reproductive toxicology, updates on carcinogenesis studies, and the evaluation of several alternative test systems for carcinogenic and noncarcinogenic effects. New scientific activities and directions of the BSRC/NIHS, NIEHS, and the U.S. National Toxicology Program (NTP) were also discussed.

## Reproductive and Developmental Toxicology

A number of available strategies to evaluate the potential reproductive and developmental toxicity of chemicals was discussed

and evaluated (4–6). Until recently, emphasis in reproductive toxicity testing has been on the male. However, in the NTP database of nearly 100 chemicals tested for reproductive toxicity, no chemical has demonstrated only male reproductive toxicity. All chemicals were either only toxic to the female reproductive system or to both the male and female (e.g., phthalate esters and glycol ethers). Yet, over the last 10 years there have been numerous reports examining the site and mechanism of action of these chemicals in the male with virtually no studies on the effects of these chemicals in females.

An analysis of reproductive toxicity studies over the past several years indicated that there were very few endpoints measured in females compared to males in reproductive toxicity studies. Also, there was not a published, logical, integrated approach to defining the site and mechanism of action of female reproductive toxicants as had been developed for the male. Furthermore, the use of ovarian histopathology lagged severely behind that of testicular histopathology. In the male, the first reproductive toxicity test, because of the numerous endpoints measured, can often provide significant clues about the identity of the target organ and lead directly to an informed pathogenesis study. For the female, however, often the only information obtained in this type of study is a decrease in the number of pups, or perhaps a change in the estrous cycle. Because the estrous cycle can be normal in the presence of significant adverse reproductive effects in the female, this is considered an insensitive endpoint. Thus, there are often few clues as to the target site in the female. NTP scientists have devised a logical approach to this problem (Fig. 1). The approach includes the following measurements: crossover mating to determine affected sex, implantation sites, corpora lutea, live and dead pups, ovarian weight, and ovarian histology. It could be used as either the first assessment of female toxicity or as a second-tier test, after the initial reproductive toxicity assessment. This approach should lead the investigator to the target organ(s) of the toxicant. The rationale for the design is as follows:

Fewer live pups in treated females (mated with untreated males) indicates a female reproductive toxicant; altered

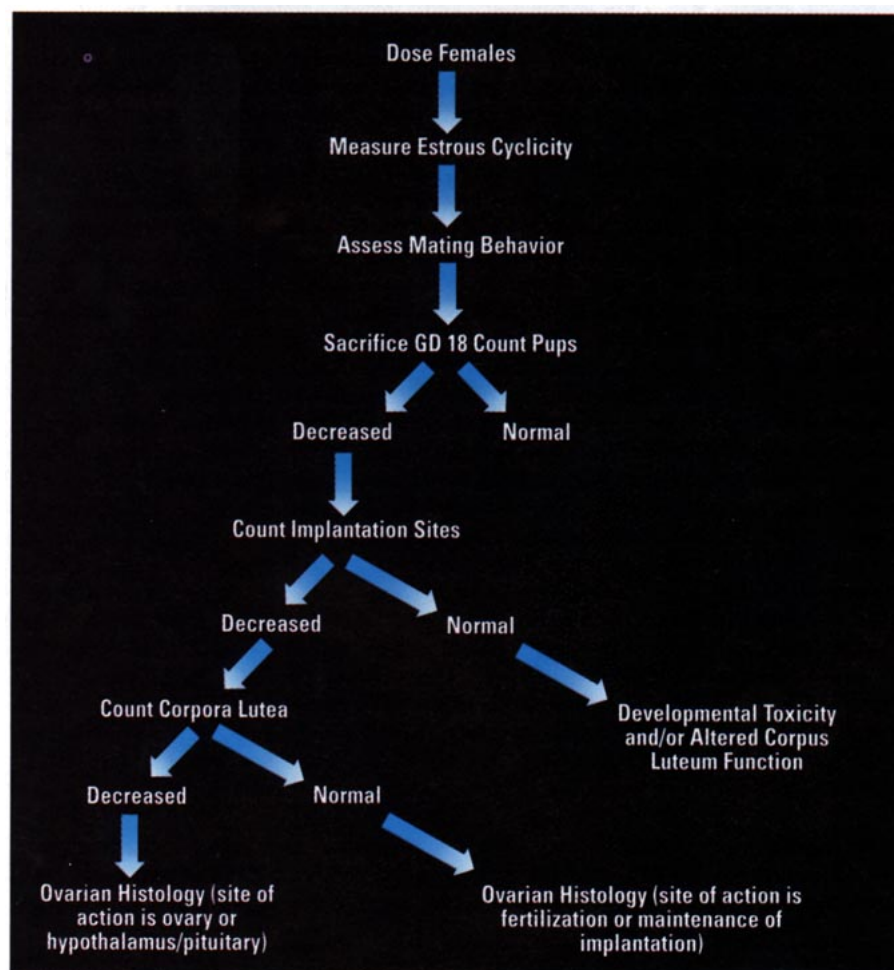


Figure 1. Schematic for initially identifying the location of reproductive toxicity in females. GD, gestation day.

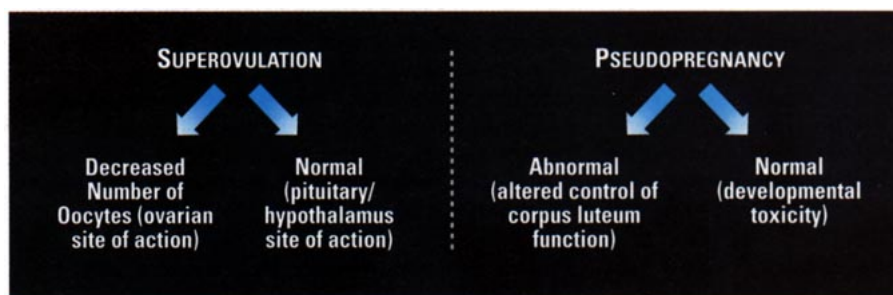
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estrous cyclicity and/or altered mating behavior would substantiate this conclusion. If there are fewer live pups but a normal number of implantation sites, then the effect occurs after implantation. This would probably be the result of genetic toxicity (dominant lethal mutation), developmental toxicity to the conceptus/fetus, or lack of sustained and adequate corpus lutea function, leading to hormonal insufficiency.

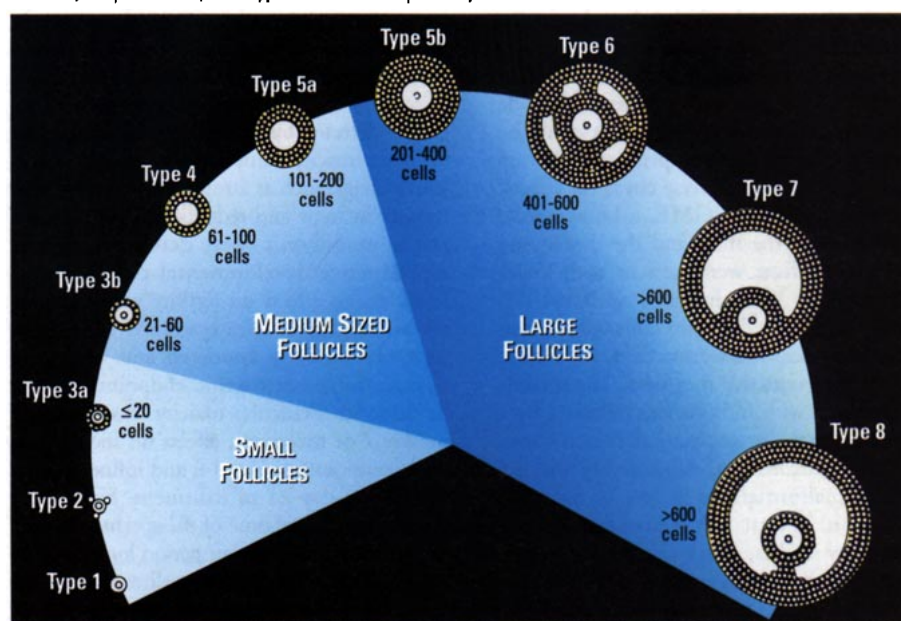
If the number of implantation sites is decreased, then there is either a decrease in the number of ovulated oocytes, or there are effects on fertilization and/or implantation. If the number of corpora lutea is normal, this suggests alterations in the oocyte, resulting in impaired fertilization/implantation. If the number of corpora lutea is decreased, superovulation can theoretically be used to separate an effect on the ovary from an effect on the central hormonal control tissues [hypothalamus and pituitary (H/P)]. In theory, superovulation should be able to separate a direct ovarian site of action from an indirect ovarian effect, but this procedure has not been assessed for this use (Fig. 2A).

In the event that the number of corpora lutea is normal (and implantation sites are fewer), the superovulation test is not needed because the ovary is shedding adequate numbers of oocytes. Instead, that group of animals may be used for a pseudopregnancy test (Fig. 2B). Again, this test has not been used for this application, but it should suit the need, in theory. A normal pseudopregnancy test implies the successful development of a functional corpus luteum, which depends on luteinizing hormone and prolactin from the pituitary. However, because pseudopregnancy is shorter than pregnancy (12–14 days versus 19–21 days), and because the animals are intentionally sacrificed after 9 days in the pseudopregnancy test, problems that developed later in gestation would not be identified by this test.

If the pseudopregnancy test is abnormal, it indicates a uterine, corpus luteal, or H/P site of action. Thus, using this type of approach should identify the site of toxicity as the ovary, H/P, fertilization, implantation, or a combination of these. Ovarian histology where one counts the number of small, growing, and large follicles, the percentage of each of these that is atretic, and the percentage of dividing cells per follicle can also be very useful in defining the site of action of a female reproductive toxicant (Fig. 3). The assessment of estrous cyclicity and mating behavior can provide additional evidence supporting a proposed site of action. Further experiments will then determine the target cell within that tissue (i.e., pathogenesis study) and explore pos-



**Figure 2.** Tests that would be added to a fertility test to aid in the determination of the site(s) of action of a female reproductive toxicant. The superovulation test distinguishes a direct ovarian action from that on the hypothalamus and pituitary. This test is appropriate when one finds fewer live pups and fewer corpora lutea in treated females. The pseudopregnancy test is indicated when there are fewer live pups, with a normal number of implantation sites. It distinguishes development toxicity from toxic effects on the uterus, corpora lutea, and hypothalamus and pituitary.



**Figure 3.** Follicle classification scheme. Follicles are classified as small, medium (growing), or large (antral) according to this scheme based on their size and number of granulosa cells.

STUDY DAYS	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28				
MALES	Co	Co	Co											Co	Co	Co	Co	Co	Nec
FEMALES (a)							Co	Co	Co	Co	Co						Nec		
FEMALES (b)	Co	Co	Co	gd	6	7	8	9	10	11	12	13	14	15	Bi	Nec			
					6	7	8	9	10	11	12	13	14	15	Bi	Nec			
						6	7	8	9	10	11	12	13	14	15	Bi	Nec		

**Figure 4.** Subchronic toxicity screen including observations for reproductive and developmental toxicity. Co, cohabit; gd, gestation day; Bi, birth; Nec, necropsy. Shaded areas indicate days of chemical dosing. Parameters of the screen were as follows: subchronic toxicity and male infertility: three doses plus control,  $n = 10$ /group; body weights taken on days 3, 7, 11, 15, 19, 23, 28; modified functional observational battery; gross necropsy; histopathology on liver, kidney, testis; hematology on blood at termination; fertility before and during chemical administration, epididymal sperm motility and total epididymal sperm count. Females (a): subchronic toxicity and female fertility, three doses plus control,  $n = 10$ /group; body weights taken on days 0, 4, 8, 12, 16, 20, 24, 28; percent pregnant; number of implants; number of corpora lutea. Females (b): developmental toxicity, three doses plus control,  $n = 10$  smear-positive females/group; confirm mating by vaginal smear or plug during cohabitation; dose females on gd 6–15; body weights taken on days 0, 6, 10, 15, and postnatal days 1 and 4; number and weight of pups at postnatal days 1 and 4; uterine implantation sites at necropsy and postnatal day 4.

sible mechanisms of action. Details on setting up an ovarian pathogenesis study have recently been published (7).

### Short-Term Reproductive/Developmental Toxicity Screen

Several short-term tests for reproductive and developmental toxicity to provide preliminary screening data on the toxicity of chemicals for which little or no data exist were evaluated. These included tests recommended by the Organization for Economic Cooperation and Development (OECD) and short-term tests conducted by the NTP.

Data were presented from work done at NIEHS using a 21-day test for reproductive and developmental toxicity that also incorporates general toxicity endpoints (Fig. 4). Studies on ethylene glycol monomethyl ether (EGME) (8) and indium trichloride (9) indicate that a short-term test could be useful in identifying the processes that are most vulnerable to adverse chemical effects. For example, with EGME, a known toxicant for both the male and the developing organism, effects were seen in both males and females. With indium, the fetus was primarily at risk: dead implants were found in the absence of adverse effects on female or male reproductive measures. The preliminary study with indium was followed by a conventional developmental toxicity test, which confirmed fetal vulnerability and the lack of malformations in soft or hard tissues. This, in turn, was followed by *in vitro* teratology studies, which demonstrated toxicity directly to the conceptus at doses as low as 50  $\mu$ M (9). Long-term definitive studies are planned with indium to verify the degree and types of chronic toxicities that were missed by the short test, if any. This design points out the utility of using two groups of females dosed differently: one during gestation only and the other dosed during ovulation and through the first part of development (9). Data from these groups together can be used to identify which part of reproduction in females is primarily affected: reproduction (ovulation, fertilization, implantation) or development (fetal development after implantation). This is a property unique to this design and is not found in other short-term test designs. This design is also now being used by the NTP for rats, incorporating two changes: 5 days (versus 3 for mice) of mating are used to generate the pregnant females for the gestational exposures, and the length of the entire test is increased to 28 days. Increased exposure should increase the probability of detecting toxicity. NIEHS will continue to use this test for in-house studies as needed and will incorporate components of other testing (e.g., OECD test) as appropriate.

### OECD Reproductive/Developmental Guidelines

The OECD proposed the guideline "Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test" (Repro/Tox) for rapid screening of high production volume chemicals in 1990 (10). NIEHS/BSRC scientists have used this protocol, designed to detect general toxicological effects simultaneously with reproductive/developmental effects (11). A number of chemicals have been screened using this protocol (12), and an ad hoc expert meeting on "Reproductive Toxicity Screening Methods" was held at BSRC, NIEHS, in October 1992 to evaluate the Repro/Tox protocol. The Repro/Tox protocol is a very useful preliminary screening test, but it also has several shortcomings. Studies on nitrobenzene were conducted to examine whether disturbances of fertility associated with testicular toxicity can be detected by this screening test. In the systemic toxicity component, histopathological findings, such as atrophy of seminiferous tubules in testis and reduction of spermatozoa in epididymis, were detected. In the reproductive/developmental component, however, no effects on fertility were detected. Subsequent studies examined when the testicular toxicity appeared and whether changes in spermatogenic endpoints could be detected. Testicular toxicity was detected on day 7 of treatment; effects on spermatogenic endpoints on day 14; and influence on fertility on day 21 of treatment. Since the epididymal transit time of the sperm is more than 10 days, a treatment period longer than 14 days is necessary to detect disturbances of male fertility due to testicular effects. To evaluate male fertility and sperm endpoints, examination at 28 days after initial dosing is recommended because this will make it possible to compare the results with those of 28 repeated-dose toxicity tests.

### Testicular Toxicology

The complex interactions of the various cells of the testis make it especially difficult to understand testicular toxicity. Histological evaluations of testicular lesions induced by adriamycin, cyclophosphamide, nitrobenzene, ethane-1,2-dimethane sulfonate, ethylene glycol monomethyl ether, and cadmium chloride strongly suggest that early changes, rather than those occurring at late stages, are of particular assistance in understanding the mechanisms of action of these chemicals (13).

### Alternative Tests

**Alternative models to evaluate developmental toxicity.** In response to the large number of chemicals that require testing for developmental toxicity, *in vitro* systems have been developed to serve as screening tools. The time and cost-effectiveness of

such *in vitro* systems may allow them to be used as screening systems to help establish priorities for further assessment of developmental toxicity *in vivo*. One approach to developmental toxicity hazard assessment is the use of whole embryo screening tests. Teratogenesis screening assays using whole embryos have the potential to detect disruptions to the cellular mechanisms and cell-cell interactions critical for the normal process of development. Because of the complexity of development, an intact embryo may be better at predicting developmental toxicity as compared to individual cell types. Two such whole embryo systems involve the use of embryos of the *Drosophila melanogaster* and the South African clawed frog, *Xenopus laevis*.

**Drosophila.** Exposure of *Drosophila* during developmental morphogenesis to mammalian developmental toxicants has indicated that this whole embryo system has the potential for future use as a screening system. The assay includes malformation of the embryo as a distinct measure of effect, separate from survival and general growth endpoints. There are numerous structural endpoints to examine in the embryo potentially limiting its practical usefulness as a screening device. In an attempt to focus the system on a particular endpoint or group of endpoints, previous studies conducted in collaboration with the National Institute of Occupational Safety and Health identified an abbreviated assay. It was shown that the predictive value of the assay is maintained if limited to the evaluation of the distinct endpoint of bent humeral bristles. Based on these findings, NIEHS is in the process of establishing a validation study for the *Drosophila* as a model system to screen chemicals for their potential mammalian teratogenic hazards (14).

**FETAX.** The frog embryo teratogenesis assay-xenopus (FETAX) is a 96-hr whole embryo developmental toxicity test that uses embryos of the South African clawed frog, *Xenopus laevis* (15). Embryos are exposed to the test chemical continuously from the early blastula to the stage 46 free-swimming larvae. During this time they undergo cleavage, gastrulation, and organogenesis similar to mammals. These processes involve all of the developmentally important cellular events, including cell division, interaction, migration, differentiation, and death. In contrast to other short-term or *in vitro* screens, FETAX endpoints can potentially detect all four manifestations of mammalian developmental toxicity: growth retardation, structural malformations, death, and functional deficits. Functional deficits are detected by assessing locomotor activity and sensory reflex responses and provide a limited eval-



uation of the neural network. Teratogenicity has been detected for a variety of chemicals and complex mixtures, including selenium (16), aromatic amines (17), and complex shale oil mixtures (18).

To increase the predictive accuracy of the FETAX system to detect mammalian developmental toxicants that require metabolic activation, an exogenous metabolic activation system was developed (19,20). This system uses the addition of Aroclor 1254- or Isoniazid-induced rat liver microsomes, which convert proteratogens such as cyclophosphamide to an active teratogenic form. A multilaboratory validation study is currently being conducted by the NTP to further determine the repeatability and reliability of the FETAX system as a developmental toxicity screening test. A total of 20 coded chemicals that have been tested by the NTP in rodents will be used in the validation study including both strong and weak teratogens, nonteratogens, and teratogens that require metabolic activation. Studies are being conducted by other laboratories to elucidate the mechanisms by which chemicals cause developmental abnormalities in the FETAX system.

Preliminary validation tests have demonstrated the potential of each of these embryo systems to identify hazard and have stimulated additional studies to establish data to meet the criteria for *in vitro* teratogenesis assay validation.

**Postimplantation Embryo Culture.** The usefulness of the *in vitro* test using postimplantation rat embryo culture to evaluate developmental toxicity of low levels of environmental chemicals was discussed. 2-Chlorodibenzofuran, a contaminant in chlorinated tap water, and selenium were found to be teratogenic using this *in vitro* test (21,22). The use of this *in vitro* culture of postimplantation embryos has several advantages: 1) it requires only small amounts of test chemicals; 2) embryotoxicity similar to that observed *in vivo* has been demonstrated for several chemicals; and 3) maternal toxicity is eliminated.

The whole embryo culture system has many benefits in determining the direct effects of an agent, such as precise embryonic staging at the time of exposure, and ability to control the concentration of test agent. However, the lack of maternal metabolizing and excretion systems may limit the direct extrapolation between effects produced *in vitro* and those observed *in vivo*. Ethanol is an example of an agent that is rapidly metabolized and eliminated *in vivo*. In fact, after a teratogenic dose of ethanol *in vivo*, the parent compound only has a 2- to 6-hr half-life in the maternal serum (23). However, when the effects of ethanol have been evaluated *in*

## FOURTH UNITED STATES-JAPAN MEETING ON THE TOXICOLOGICAL CHARACTERIZATION OF ENVIRONMENTAL CHEMICALS: PARTICIPANTS

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*vitro*, most studies have used 24- to 48-hr exposure periods. NTP scientists evaluated the developmental toxicity of ethanol *in vitro* using concentrations and exposure periods that mimicked those that were teratogenic *in vivo*. The effects of ethanol *in vitro* were characterized by craniofacial dysmorphogenesis and growth retardation. The incidence and severity of these effects were highly dependent on the concentration and length of exposure. For example, to induce neural tube closure defects, an 8- to 12-hr exposure period to a high ethanol concentration (600–800 mg/dl) was required (24). These studies further indicate that dispositional information must be considered when assessing the toxicity of an agent *in vitro*.

The culture system has also been used to study embryonic metabolism and nutrition at the early neurulation stage. It is currently accepted that the predominate route of energy production is from the glycolytic metabolism of glucose (25–27). Using specific metabolic inhibitors, recent work indicates that mitochondrial metabolism appears to be important for normal development and energy production. Continuing work will focus on understanding the apparent lack of glucose utilization by the Krebs cycle and the identification of the substrates used for the Krebs cycle and oxidative phosphorylation.

### Carcinogenesis Studies

**Update on NTP efforts.** The NTP has recognized the importance of incorporating additional mechanistic studies into chronic toxicity/carcinogenicity assays and has initiated a number of collaborative ties with other researchers to incorporate mechanistic studies. For example, in collaboration with a nonprofit research institute, eight investigators were provided rats from the NTP ozone study for their research. These studies provide functional, structural, and biochemical studies on the NTP ozone-exposed animals. These mechanistic studies will be reported in conjunction with the basic NTP studies and will provide crucial and timely data on ozone, as the United States is currently reevaluating the standards on ozone. Dibutyl phthalate, an

important U.S. Clean Air Act chemical (and hazardous waste chemical), is also being studied in collaboration with NIEHS intramural scientists to provide the necessary mechanistic information. Dibutyl phthalate is of special interest to NTP and NIEHS scientists because it is found in the blood of most people, causes proliferation of peroxisomes in animals, appears to act through a receptor, and can provide useful mechanistic data that may be applicable to other chemicals. Carbon disulfide, another important U.S. Clean Air Act chemical, is being studied in collaboration with investigators from the Health Effects Research Laboratory, Environmental Protection Agency, and with the extramural NIEHS program to provide the mechanistic information on the neurotoxicity of this chemical.

**Update on NIEHS/BRC efforts.** Urinary bladder carcinogenesis from melamine has been reported to occur in rodents with urolithiasis. Studies on the relationship between urolithiasis and urinary bladder carcinogenesis revealed that sodium chloride supplementation in the diet inhibited not only calculus formation but also the hyperplastic lesions and tumors induced by melamine. These results suggest that the tumors and hyperplastic lesions of the urinary bladder were secondary to calculus formation and not directly attributable to the biological effects of melamine. Similarly, the chemical bisacodyl induces both calculi and epithelial proliferative lesions in the urinary bladder of male rats, and studies indicate that bisacodyl-induced proliferative lesions were not caused directly by bisacodyl but were secondary to calculus formation (29).

Studies to compare the *in vitro* cytotoxicity and *in vivo* tissue responses induced by natural rubber latex materials were evaluated. The *in vivo* implantation test showed that among 13 histological parameters, thickness of the inflammatory layer was the most useful index to evaluate tissue responses quantitatively. A comparison of two *in vitro* cytotoxicity assays (colony assay using V79 cells and the agar diffusion assay with L929 cells) showed that the colony assay provided a more reliable prediction

of the tissue response than the agar diffusion assay (30).

Several problems with the evaluation of rodent carcinogenicity in endocrine organs were presented. Potassium pyrophosphate, a food additive, induced tubular necrosis in kidneys of treated female F344 rats (2.5 and 5% in diet) and high-dose male rats. Chronic nephropathy and diffuse hyperplasia of the parathyroid gland and fibrous osteodystrophy in bone were also observed in high-dose females. These changes suggest the onset of persistent hypercalcemia, which may cause adrenal medullary proliferative lesions (31). The increased incidence of pheochromocytomas and of adrenal medullary proliferative lesions in treated females may be attributable to the persistent hypercalcemia and should be considered secondary to severe, prolonged renal toxicity.

Analysis of cell proliferation in spontaneous proliferative lesions in the adrenal medulla of F344 rats used in 2-year carcinogenicity studies was performed by determining labeling indices for proliferating cell nuclear antigen (PCNA), mitotic indices, and numbers of silver-stained nucleolar organizer regions (AgNORs) (32). The lesions were classified into hyperplasia, well-differentiated medullary tumor (WMT), atypical medullary tumor showing cellular pleomorphism and atypia (AMT), and malignant medullary tumor with invasion or metastasis (MMT) categories. The PCNA labeling index and the number of AgNORs in proliferative lesions of the rat adrenal medulla exhibited a stepwise increase from normal medullary cells through WMT, AMT, and MMT. These data indicate that the AMT cannot be definitely classified, despite its marked cellular atypia, as one of the malignant or metastatic tumors.

Thyroid follicular proliferative lesions were induced in male F344 rats treated with high and low doses of thiourea (TU) and sulfadimethoxine (SDM) for 20 weeks. However, rats treated with TU or SDM did not reveal any consistent changes in plasma  $T_3$ ,  $T_4$ , and thyroid-stimulating hormone (TSH) levels, except for high-dose TU group, which showed decreased plasma  $T_3$  and  $T_4$  and increased TSH. Prolonged stimulation of the thyroid-pituitary feedback mechanism, which results in elevation of plasma levels of TSH, leads to thyroid tumors (33). However, consistent changes in thyroid hormones and TSH levels were not found in rats treated with TU and SDM for 20 weeks. Therefore, the present studies may suggest that prolonged stimulation of the thyroid gland by excess of TSH is not always needed for the promotion and progression of thyroid proliferative lesions.

Brain tumors induced by transplacental application of ethylnitrosourea (ENU) in F344 rats mainly consisted immunohistochemically of two distinct types of vimentin-expressing cellular nests termed "perivascular small cellular nest" (PSCN) and "large cellular nest" (LCN). PSCNs contained cells expressing glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), and low-affinity nerve growth factor receptor (LNGFR). LCNs contained cells showing a neuronal phenotype that expressed low- and middle-molecular-mass neurofilament proteins as well as NSE and LNGFR. Topographically, bidirectional cell transitions from PSCNs to astrocytes and LCNs was discerned. The present study suggests that ENU-induced "gliomas" (34) originate from pluripotent germinal neuroepithelium and that PSCN plays a role as cell source both for astrocytic and neuronal lineages.

The NIEHS/BSRC is conducting carcinogenicity tests on the following chemicals: Trp-P-2, potassium pyrophosphate, ferric chloride, melamine, tannic acid, trimethyl cyclohexane, cyanoguanidine, sevioside, cyclodextrin, *n*-paraffin, potassium iodide, histidine, jasamycin. Chemicals tested for chronic or subchronic toxicity are: rare earth metals, *N,N*-dimethylethanolamine,  $\alpha$ -methylbenzylphenol, tributyoethyl phosphate, *p*-methylbenzenesulfonmethyldiiodide.

### Using Fish to Evaluate Chemical Carcinogens

In the last 15 years, the use of small fish species in carcinogenicity testing has gained momentum. The small fish model offers many advantages as a bioassay test system: 1) Significant savings in cost and time over rodent studies because large numbers of animals can be maintained in a limited area and certain species of the teleost family are hardy and easily bred under standardized laboratory conditions. 2) Inbred strains with low spontaneous tumor rates have been developed that will help reduce the variability in tests; these strains have effectively demonstrated the carcinogenic effects of certain chemicals under experimental conditions in a shorter time period than necessary for rodent studies (35-37). 3) When used as a screening system, aquatic studies can provide toxicology information that could lead to fewer and better designed studies in rodents. Toxicokinetic studies can be designed for aquatic animals (38). Determining the level of test chemical or its metabolites in biological samples can be used to determine bioavailability, to select doses, and to correlate toxic effects with systemic availability. 4) Physiological features of aquatic organisms (i.e., total exposure in an aque-

ous environment and the aquatic gill system have similarities to the more costly rodent inhalation studies) provide unique opportunities for certain mechanistic studies. There are also natural advantages for evaluating chemical mixtures and chemicals found in water (39).

In December 1991 a "Workshop of Carcinogenesis Testing with Fish" was held at the National Institute of Environmental Health Sciences to evaluate the utility of the small fish model. NIEHS is currently planning to conduct long-term (16-month exposure) studies in two species of small fish which will provide an excellent opportunity to combine the advanced fish technology with the established, exacting study conduct and reporting requirements of an NTP 2-year rodent carcinogenicity study. Three compounds will be tested, each of which has been previously indicated to be a carcinogen in the NTP rodent model. This will help evaluate the utility of the small fish model in hazard characterization and how it can be related to rodent studies.

### Conclusion

The U.S. and Japanese participants agreed that the data presented during the meeting on reproductive toxicology, carcinogenesis studies, and alternative test systems was very timely and beneficial. The meeting provided a forum for the U.S. NTP and the Japanese NIEHS to design future collaborative studies on environmental chemicals of mutual interest, thus avoiding duplication of effort.

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